ORIGINAL ARTICLE

Gene Expression Profiles in Living Donors Immediately After Partial Hepatectomy—The Initial Response of Liver Regeneration

Cheng-Maw Ho,[1] Po-Huang Lee,[2,3] Yeun-Tyng Lai,[2,3] Rey-Heng Hu,[2,3] Ming-Chih Ho,[2,3] Yao-Ming Wu[2,3]*

Background/Purpose: Gene expression profiles of liver regeneration are well explored in rat models. However, there are limited relative data in humans. This study aimed to show that mRNA expression profiles change immediately after right hepatectomy in living-related donors and correlate with mechanisms of liver regeneration reported in the literature.

Methods: Prospective study was conducted from March 2003 to August 2004. Living-related donors who donated right lobe of liver were included. Liver biopsies were performed at the beginning and, 5 hours later, at the end of liver resection. RNAs were isolated to synthesize cRNA. Oligo DNA microarray experiments were conducted and paired signal intensity ratios (Cy3/Cy5) were normalized with rank-invariant global Lowess regression analysis by taking base two logarithms. Genes whose average residuals more than 2.5-fold increased or less than –2.5-fold decreased were selected to get the most pronounced expression changes during this period.

Results: Five of 34 donors were included with qualified samples. The expression patterns of paired DNA microarray experiments were similar in five donors. A total of 28 upregulated and 14 downregulated genes were collected. Acute-phase proteins (serum amyloid A, complement-reactive protein, heme oxygenase-1) were upregulated. Genes related to growth signal transduction (G-protein coupled receptor-30) were downregulated.

Conclusion: Gene expression profiles immediately after partial hepatectomy were reported first in humans with the techniques of oligo DNA microarray, which were compatible with the initial gene expression patterns of liver regeneration in rats. [*J Formos Med Assoc* 2007;106(4):288–294]

Key Words: gene expression profile, liver regeneration, hepatectomy

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It is well known that liver tissue has the ability to regenerate after injury. This characteristic of human liver makes living related and small-for-size liver transplantation successful.[1–3] Among rat models of liver regeneration, partial hepatectomy is a standard method for inducing or stimulating residual liver to regenerate.[4] After partial hepatectomy, approximately 95% of remaining hepatic cells, which are normally quiescent, rapidly re-enter the cell cycle.[5,6] Much is known about the molecular mechanisms underlying liver regeneration in the rat, but it is not really known whether the same regenerative mechanisms are operative in regenerating human liver. When we reviewed the

[1]Department of Surgery, National Taiwan University Hospital Yun-Lin Branch, Yun-Lin, and [2]Department of Surgery and [3]Angiogenesis Research Center, National Taiwan University Hospital and National Taiwan University College of Medicine, Taipei, Taiwan.

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***Correspondence to:** Dr Po-Huang Lee, Department of Surgery and Angiogenesis Research Center, National Taiwan University Hospital, 7 Chung-Shan South Road, Taipei 100, Taiwan.
E-mail: pohuang@ha.mc.ntu.edu.tw

literature on this topic, no human data were found. This is important because the pattern and timing of liver regeneration observed in nonhuman primate models are significantly different compared with other nonprimate species.[7] For example, the peak expression of Ki-67, a marker of liver regeneration, occurs within hours in rat models, 72 hours in dogs, and 2–3 weeks in Rhesus macaques.[7] Species differences in liver regeneration, therefore, exist.

Our study was aimed at determining changes in mRNA expression profiles immediately after hepatectomy in living donors who donated liver tissue for liver transplantation. Changes in these profiles were correlated with mechanisms for the initial phase of liver regeneration reported in the literature.

Materials and Methods

Patients

Our prospective study was conducted from March 2003 to August 2004 at National Taiwan University Hospital's transplant center. Consecutive living donors willing to donate right hepatic lobes and specimens were studied. Standard donor right lobectomy was performed in each patient. Inflow occlusion control was not performed during operation. We sampled liver specimens from the cutting edge of the remnant left lobe at the beginning of liver resection and, about 5 hours later, at the end of liver resection after the graft had been removed. All tissue specimens were immediately snap-frozen for subsequent molecular analysis. All donors gave written informed consent. The study was conducted according to the guidelines of the Institutional Review Boards at National Taiwan University Hospital.

RNA isolation and quality control

A small piece of liver tissue ($< 1\ cm^3$) was extracted by Trizol® Reagent (Invitrogen Corp., Carlsbad, CA, USA) followed by RNeasy Mini Kit (Qiagen Inc., Valencia, CA, USA). Total RNA purified was quantified by OD 260 nm using an ND-1000 spectrophotometer (NanoDrop Technologies Inc., Wilmington, DE, USA) and qualitatively by a Bioanalyzer 2100 instrument (Agilent Technologies Inc., Santa Clara, CA, USA).

Experimental design of oligo DNA microarray, hybridization and scanning

RNA (0.5 g of total) was amplified by a low RNA input fluor linear amp kit (Agilent Technologies Inc.) and labeled with Cy3 or Cy5 (CyDye; PerkinElmer Inc., Waltham, MA, USA) during the *in vitro* transcription process. RNA from liver specimens at the beginning was labeled by Cy5 and RNA from tissue samples obtained after graft removal was labeled by Cy3. Correspondingly fragmented labeled cRNA was then pooled and hybridized to a Human 1A (version 2) oligo microarray (Agilent Technologies Inc.). After washing and drying by nitrogen gun blowing, microarrays were scanned with an Agilent microarray scanner (Agilent Technologies Inc.) at 535 nm for Cy3 and 625 nm for Cy5.

Data and statistical analysis

Signal intensities were quantified by feature extraction software (Agilent Technologies Inc.). Paired signal intensity ratios (Cy3/Cy5) were normalized with rank-invariant global Lowess regression analysis by taking base two logarithms. Using transformed data derived from each pair of competitive hybridization images, we drew scatter diagrams to compare paired signal intensity ratios for different donors, and executed Pearson product-moment correlation analysis. The given residuals explained the logarithmic gene expression ratio. We, therefore, selected genes whose average residuals were more than 2.5-fold increased or less than −2.5-fold decreased to get the most pronounced expression changes during this period.

Results

Five of 34 donors were included with the samples for qualitative analysis. The demographic data of the five donors are shown in Table 1. Similar

gene expression patterns were found, as noted in Figure 1. A total of 28 upregulated genes that showed increases greater than the 2.5 mean $\log_2$ ratio expression and 14 downregulated genes that showed decreases less than the –2.5 mean $\log_2$ ratio expression were collected. They are categorized in Table 2 based on Gene Ontology Consortium protocols. Gene names, systematic names and description are listed in Table 3 in alphabetical order. Acute-phase proteins such as serum amyloid A1 (SAA1), A2 (SAA2), complement-reactive protein (CRP), and heme oxygenase-1 were upregulated. Thyroid hormone responsive, the adjuvant in the process of liver proliferation (Figure 2), was downregulated. Genes related to growth signal transduction such as G-protein coupled receptor-30 were upregulated as was suppressor of cytokine signaling 3.

Table 1. Demographic data of the five donors

Donor	Age (yr)/sex	Donated liver volume/whole original liver volume (mL)
1	24/M	1050/1851
2	24/M	800/1481
3	46/F	550/1156
4	35/M	790/1528
5	25/M	700/1518

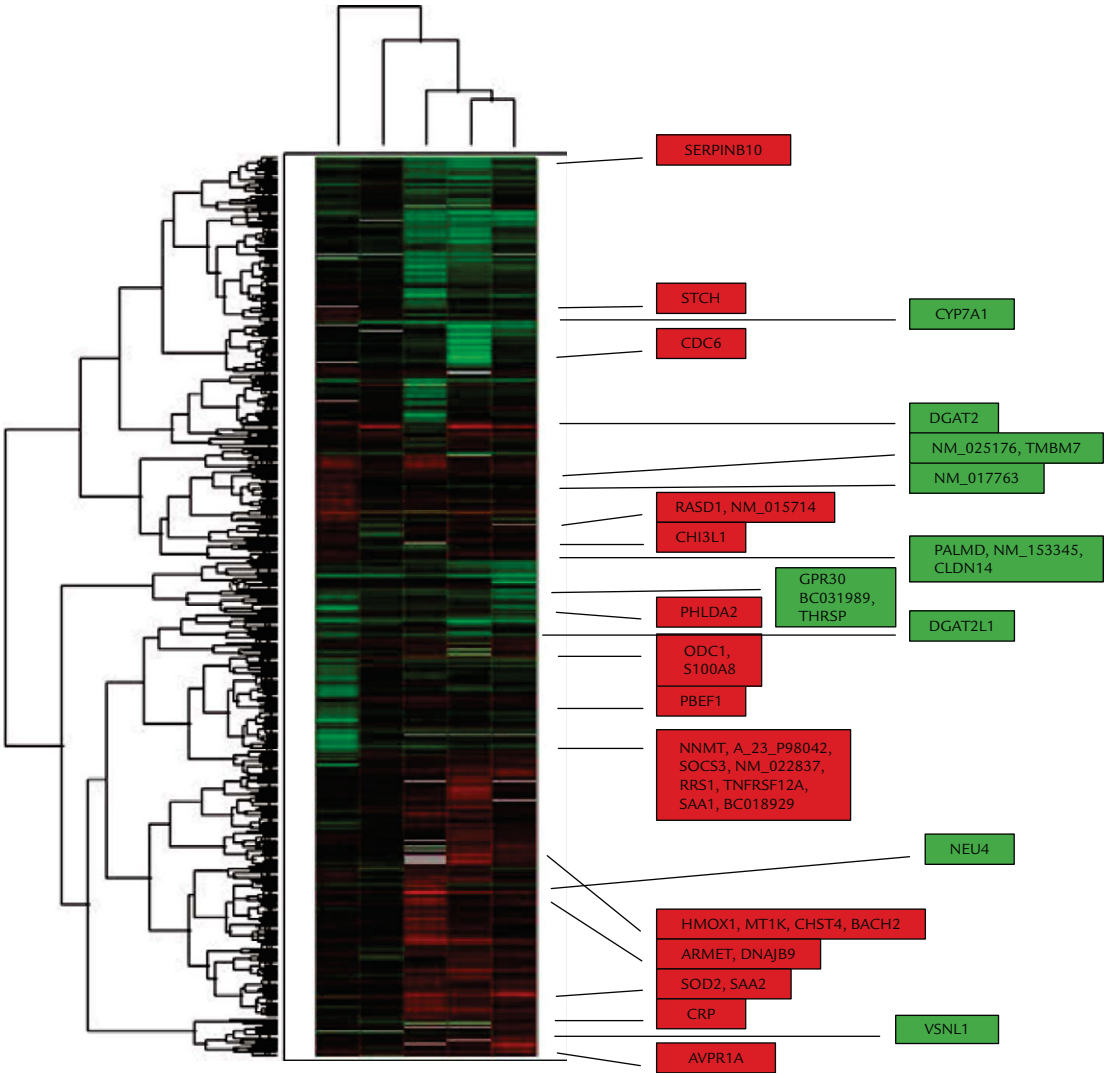

Figure 1. The different rows represent gene expression patterns in different donors. Red represents upregulated genes and green represents downregulated genes. Similar patterns of gene expression are noted in cluster analysis. Specific up- and downregulated genes are labeled as shown.

Table 2. Up- and downregulated genes category

Category	Upregulated gene names (log_2 ratio expression)	Downregulated gene names ($-log_2$ ratio expression)
Immune response	SAA1 (3.42), SAA2 (3.40), CRP (3.42), CHST4 (2.84), S100A8 (2.62), SOD2 (2.57)	–
Cell growth and/or maintenance	RASD1 (3.10), PBEF1 (2.98), NM_015714 (2.95), RRS1 (2.72), SOCS3 (2.54)	–
Cell death	PHLDA2 (2.83)	–
Signal transduction	AVPR1A (2.94)	GPR30 (2.80)
Biosynthesis	ODC1 (3.20)	DGAT2 (2.51)
Catabolism	HMOX1 (2.98)	–
Alcohol metabolism	–	CYP7A1 (2.61)
Macromolecule metabolism	CDC6 (2.84), DNAJB9 (2.58)	CLDN14 (3.54), NM_017763 (2.56)
Cell motility	TNFRSF12A (2.92)	–
Regulation of nucleometabolism	BACH2 (2.79)	BC031989 (2.88), THRSP (2.63)
Unclassified	NNMT (2.99), ARMET (2.87), CHI3L1 (2.84), BC018929 (2.72), MT1K (2.71), SERPINB1 (2.59), A_23_P98042, STCH (2.51), NM_022837 (2.50)	TMEM7 (3.03), NM_025176 (2.81), VSNL1 (2.79), NEU4 (2.61), NM_153345 (2.58), DGAT2L1 (2.54), PALMD (2.54)

Discussion

Since the report of Higgins and Anderson,[4] liver regeneration has been studied mostly using rat liver resection. In studies of liver regeneration using animal models, sampling of liver tissues at serial time points after partial hepatectomy is typically collected. Although mechanisms can be inferred from these animal studies, there have been no confirmational studies of regenerating human liver. In fact, it is considered unethical to perform biopsies at several time points after partial hepatectomy because patients would be under the risk of the potential complications of those procedures with no obvious benefits to the individual patients. As one solution for resolving this dilemma, we studied the initial response of remnant liver regeneration immediately after partial hepatectomy. In our previous unpublished study of regenerated liver volume after donor partial hepatectomy, we found that smaller livers that remained after operation had greater liver regenerated volumes. We designed the current study, therefore, for live donors after right hepatectomy. Our current report is the first research study of human liver undergoing regeneration immediately after right lobectomy for living-related liver transplantation. These samples were taken from healthy volunteers with almost no confounding factors influencing liver regeneration. This study, therefore, is the first study of samples of human liver regeneration immediately after partial hepatectomy. All donors were free from morbidity with additional procedures of liver biopsy.

In this study, we found 28 upregulated and 14 downregulated genes immediately after liver resection. After several hours, over 100 immediate-early genes had been turned on and off, presumably due to complex mechanisms that have been seen in regeneration in rat liver.[8] Many of these genes account for acute phase responses. The expression of these genes is definitely related to liver resection and is seen as the initial response of liver regeneration. Taub[5] had reviewed the acute phase response as the toxic damage or stimulus to liver trigger the release of cytokines such as interleukin-1 (IL-1), IL-6, and tumor necrosis factor-α (TNF-α), which further cause the release of acute-phase proteins such as SAA, CRP, and heme oxygenase-1. The genes for production of acute-phase proteins SAA1, SAA2, CRP, and heme oxygenase 1 were upregulated. Yang et al also

Table 3. Gene names, systematic names, and description

Gene name	Systematic name	Description
Upregulated genes		
A_23_P98042	A_23_P98042	Unknown
ARMET	NM_006010	Arginine-rich, mutated in early stage tumors
AVPR1A	NM_000706	Arginine vasopressin receptor 1A
BACH2	NM_021813	BTB and CNC homology 1, basic leucine zipper transcription factor 2
BC018929	BC018929	Pleckstrin homology-like domain, family A, member 1
CDC6	NM_001254	CDC6 cell division cycle 6 homolog (*S. cerevisiae*)
CHI3L1	NM_001276	Chitinase 3-like 1 (cartilage glycoprotein-39)
CHST4	NM_005769	Carbohydrate (N-acetylglucosamine 6-O) sulfotransferase 4
CRP	NM_000567	Complement-reactive protein, pentraxin-related
DNAJB9	NM_012328	DnaJ (Hsp40) homolog, subfamily B, member 9
HMOX1	NM_002133	Heme oxygenase (decycling) 1
MT1K	NM_005950	Metallothionein 1K
NM_015714	NM_015714	Putative lymphocyte G0/G1 switch gene
NM_022837	NM_022837	Hypothetical protein FLJ22833
NNMT	NM_006169	Nicotinamide N-methyltransferase
ODC1	NM_002539	Ornithine decarboxylase 1
PBEF1	NM_005746	Pre-B-cell colony enhancing factor 1, transcript variant 1
PHLDA2	NM_003311	Pleckstrin homology-like domain, family A, member 2
RASD1	NM_016084	RAS, dexamethasone-induced 1
RRS1	NM_015169	RRS1 ribosome biogenesis regulator homolog (*S. cerevisiae*)
S100A8	NM_002964	S100 calcium binding protein A8 (calgranulin A)
SAA1	NM_000331	Serum amyloid A1, transcript variant 1
SAA2	NM_030754	Serum amyloid A2
SERPINB1	NM_030666	Serine (or cysteine) proteinase inhibitor, clade B (ovalbumin), member 1
SOCS3	NM_003955	Suppressor of cytokine signaling 3
SOD2	NM_000636	Superoxide dismutase 2, mitochondrial
STCH	NM_006948	Stress 70 protein chaperone, microsome-associated, 60 kDa
TNFRSF12A	NM_016639	Tumor necrosis factor receptor superfamily, member 12A
Downregulated genes		
BC031989	BC031989	Thyroid hormone responsive (SPOT14 homolog, rat)
CLDN14	NM_144492	Claudin 14 (CLDN14), transcript variant 1
CYP7A1	NM_000780	Cytochrome P450, family 7, subfamily A, polypeptide 1
DGAT2	NM_032564	Diacylglycerol O-acyltransferase homolog 2 (mouse)
DGAT2L1	NM_058165	Diacylglycerol O-acyltransferase 2 like 1
GPR30	NM_001505	G protein-coupled receptor 30
NEU4	NM_080741	Sialidase 4
NM_017763	NM_017763	Hypothetical protein FLJ20315
NM_025176	NM_025176	KIAA0980 protein
NM_153345	NM_153345	Hypothetical protein FLJ90586
PALMD	NM_017734	Palmdelphin
THRSP	NM_003251	Thyroid hormone responsive (SPOT14 homolog, rat)
TMEM7	NM_031440	Transmembrane protein 7
VSNL1	NM_003385	Visinin-like 1

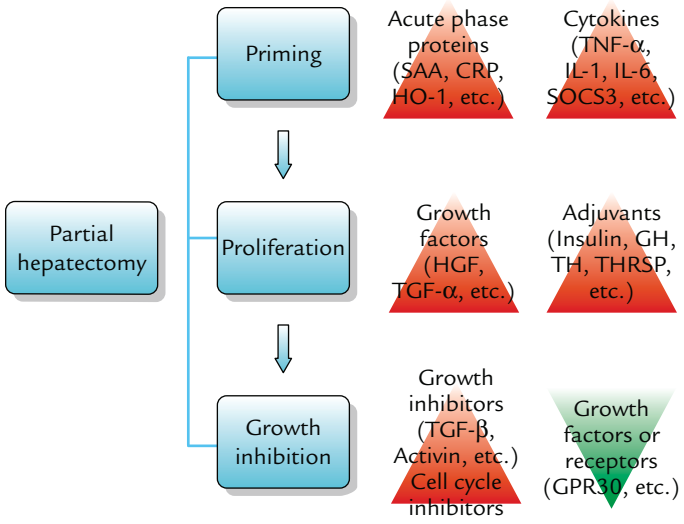

Figure 2. Sequential phases and factors involved in liver regeneration. Acute-phase proteins and cytokines, mainly serum amyloid A, complement-reactive protein, heme oxygenase-1 (HO-1), tumor necrosis factor-α, interleukin-1 (IL-1), IL-6, etc., act on hepatocytes after partial hepatectomy in the initial priming phase. Growth factors, such as hepatocyte growth factor and transforming growth factor-α, act on primed hepatocytes to make them through the cell cycle and undergo DNA replication. Insulin, thyroid hormone, and growth hormone act as adjuvants for liver regeneration. The factors that determine the termination of cell replication are not known but are likely to involve cell cycle inhibitors, turn-off of growth factor production, and downregulated growth factor receptor. SOCS3 = suppressor of cytokine signaling 3; THRSP = thyroid hormone responsive; GPR30 = G protein-coupled receptor 30. [Modified from Reference 10]

reported that overexpression of heme oxygenase-1 potentiates the survival of small-for-size liver grafts in rats.[9] These proteins help protect against damage to the liver and are prerequisites for liver regeneration. We can infer that rapid signal information flows from the insult to the expression of responsive proteins during the resection period. Interestingly, the genes related to signal transduction, such as G protein-coupled receptor 30 (GPR30), were downregulated after liver resection. The gene for suppressor of cytokine signaling 3 was upregulated. We know that liver regeneration in rat models is composed of three sequential phases: priming, proliferation, and growth inhibition (Figure 2). The acute phase response is part of the priming phase. The second phase of liver regeneration, the proliferative phase, needs adjuvants such as norepinephrine, insulin, thyroid hormone, and growth hormone for cell proliferation.[10] In our study, the gene for thyroid hormone responsive was downregulated. These findings suggest that there might be a fast feedback inhibition response as the signal cascade of liver regeneration is turned on and then passes by.

Liver regeneration is crucial for living-related liver transplantation. Failed regeneration would result in postoperative morbidity and mortality. In the study by Cho et al, a remnant liver volume of < 35% does not appear to be a contraindication for right liver procurement from living donors.[11] A second insult, however, in the setting of relative hepatic insufficiency after liver donation could initiate a potential fatal cycle of complications.[11,12] Understanding the genes involved in liver regeneration should clarify the "physiology" of liver regeneration and ultimately improve the regeneration process and further reverse pathologic processes.

GPR30, a member of the G protein-coupled receptor 1 family, encodes a multi-pass membrane protein that localizes to the endoplasmic reticulum. The protein binds estrogen, resulting in intracellular calcium mobilization and synthesis of phosphatidylinositol 3,4,5-trisphosphate in the nucleus.[13] It was considered to be a potential signaling pathway in breast carcinogenesis.[14,15] The significance of GPR30 in liver regeneration was unknown. The stimulatory effects of estrogen on cholangiocyte proliferation in bile duct ligated rats involve the activation of the Src/Shc/ERK signaling cascade.[16] GPR30 may play a potential significant role in cross-talk between estrogen and the mitogen-activated protein kinase pathway in bile duct regeneration after partial hepatectomy.

There are some limitations to our study. There are changes that we may have missed in our study. For example, IL-1 and IL-6 were upregulated. However, they were not upregulated as much as 2.5 mean $\log_2$ ratio. Hepatocyte growth factor activator, which is necessary for efficient liver regeneration,[17] was downregulated in our study, which further supported the hypothesis of fast

feedback inhibition discussed previously. The mean $\log_2$ ratio for this molecule, −1.17, also did not meet our selection criteria. Moreover, we sampled liver only at two time points due to ethical difficulties of specimen sampling after the operation is complete. Serial gene expression profiles, which can be shown well in animal studies,[18–20] cannot be shown here, again because of ethical issues. Animal models involve removal of two-thirds of the liver for regeneration, while in our study, a mean proportion of 49.8% of human livers remained (for the safety of our donors).

Transplanted liver in the recipient regenerated faster than remnant liver in the donor with regard to liver volume,[2] but we have no data about gene expression profiles in human grafts. Moreover, many unknown and unclassified genes were excluded just because they were "unknown", which means there is still a long way to go before we fully understand human mechanisms of liver regeneration.

Our report contains the first gene expression profiles of human liver tissue after partial hepatectomy. Acute-phase genes were upregulated, while genes related to growth signaling were downregulated. Further study is needed to investigate changes in other genes related to liver regeneration in human.

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